

Organic acids induce by metal-tolerant *Pantoea* sp. WP-5 and biogas residues enhanced phytostabilization of cadmium in soil

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Abstract

This study investigated the phytoremediation potential of maize (*Zea mays* L.) in Cd contaminated soil through co-inoculation of metal tolerant plant beneficial rhizobacteria (MtPBR: *Pantoea* sp. strain WP-5) with organic manures (PM: poultry manure and BGR: biogas residues). The objectives of this study were to i) examine comparative efficiency of MtPBR, PM and BGR alone or in combined form to improve maize biomass and physiology, and ii) understand the role of organic acid production in root exudates of maize for Cd accumulation and translocation. *Pantoea* sp. WP-5 showed tolerance to high Cd concentration (1000 mg L^{-1}), thereby inoculated to maize seeds sown in soil spiked with 75 mg Cd kg^{-1} soil and 500 g each of the organic manures per pot. The co-inoculation of MtPBR + BGR significantly ($P < 0.05$) increased chlorophyll contents, root/shoot dry weight, photosynthetic rate, stomatal conductance and relative water contents, whereas decreased electrolyte leakage, malondialdehyde contents, ascorbate peroxidase and catalase activity in maize over the control treatment. The co-inoculation of MtPBR + BGR produced significantly ($P < 0.05$) higher concentrations of acetic and citric acid (52.7 ± 0.5 and $22.8 \pm 0.08 \mu\text{g g}^{-1}$ root fwt, respectively) in root exudates of maize, which immobilized Cd within plant roots inferred by the positive relation (root Cd vs. organic acids; $R^2 = 0.80\text{--}0.92$) and reduced Cd translocation to shoots inferred by the negative relation (shoot Cd vs. organic acids; $R^2 = 0.81\text{--}0.90$). It is concluded that the application of MtPBR + BGR enhanced organic acid induced phyto-stabilization and accumulation of Cd in roots and restricted its translocation to shoots.

1. Introduction

Soil, the only growth medium for arable crops, is now becoming useless due to deposition of heavy metals. Heavy metals (HMs) like cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), arsenic (As), zinc (Zn) and copper (Co) are frequently found in agricultural soils (Ahmad et al., 2020). The HMs are known to produce reactive oxygen species (ROS), stimulate/inhibit antioxidant enzyme production, exert oxidative damage to plants, damage cell organelles and sometime cell death occurs (Georgiadou et al., 2018; Berni et al., 2019). Moreover, the functioning of enzymes is retarded due to formation of bond between HMs and sulfhydryl groups of the enzymes (Most and Papenbrock, 2015; Sethi and Gupta, 2015).

Cd is present in soil ($0.1\text{--}0.2 \text{ mg kg}^{-1}$) and is lethal metal to living system which include plants, animals, and humans. This poisonous HM is released into the soil through number of anthropogenic activities which include mining, fertilizer and batteries production and wastewater application (Ahmad et al., 2020), and caused contamination of soil with Cd (Latif et al., 2020). Soil contamination with Cd is a major threat to agriculture, as it has negative effects on plant biomass and physiology (Ahmad et al., 2020). Plants take up Cd from soil at fast rate and transport it to leaves through xylem tissues (Pirsellova et al., 2011; Rizwan et al., 2016). Cd stress causes reduction in CO_2 conductance, which lead to overall photosynthesis inhibition (Anjum et al., 2016). Physio-chemical and biological techniques are used to

remediate heavy metal contaminated soils; however, phytoremediation is an effective technique (Hasan et al., 2019).

Maize (*Zea mays* L.) due to its better Cd accumulation capacity and high biomass production potential has been widely used as probable candidate for management of Cd- contaminated soils (Rizwan et al., 2016). Maize is resistant to moderate level of Cd stress in soil. However, seed germination, mineral nutrition, photosynthesis, and growth/yields of maize may be reduced due to higher level of Cd contamination in soil (Ahmad et al., 2020). Role of root morphology and root functions in maize Cd uptake is imperative (Bi et al., 2009; Rizwan et al., 2016) as the most of the Cd entry in maize through apical parts of the roots has been reported as compared to root basal parts (Lux et al., 2015). After absorption by the root, large proportion of the Cd is fixed in the roots while only a small amount of the Cd is translocated to shoot (Puertas-Mejia et al., 2010; Ahmad et al., 2014, 2020). Due to this reason, maize can be used as potential candidate in the phytoremediation of low- to moderately-Cd-contaminated soils (Xu et al., 2013).

Organic amendments which include use of plant beneficial rhizobacteria (PBR), manures like compost, farm yard manure (FYM), poultry manure (PM) and biogas residues (BGR) not only help in improving plant growth but also regulate the rhizosphere bioremediation of metals through improving rhizo-extraction and rhizo-immobilization processes (Khurana and Kansal 2014; Shumba et al., 2014; Ahmad et al., 2015, 2018). Application of BGR and compost has increased maize tolerance to Cd and improved the growth and biomass yield of maize in Cd contaminated soil (Ahmad et al., 2015). Use of some chemicals like EDTA, nitrilotriacetic acid, citric and oxalic acid as well as organic amendments have a significant role in bio- or phyto-remediation of heavy metals (Xu et al., 2013; Shahid et al., 2014; Ahmad et al., 2015; 2018; Hasan et al., 2019). Functional group (COOH^- as well as OH^-) of the organic acids make a complex with metals like Cd^{+2} and restrict its translocation (Xu et al., 2013; Hasan et al., 2019; Bali et al., 2020), in contrast, Chen et al. (2020) found higher accumulation of Cd in sorghum grass in the presence of citric acid and maltose. However, studies on the inoculation of organic acid producing bacteria to maize and their effect on root exudates under Cd stress is remain elusive. Production of organic acids like acetic acid, oxalic as well as citric acid by the plant beneficial rhizobacteria and the role of these acids in P solubilization has been reported in our previous studies (Tahir et al., 2013, 2015). We hypothesized that use of the organic acid producing MtPBR along with organic treatments such as BGR and PM enhance the phytoextraction and bioaccumulation of Cd in *Zea mays* L. under Cd contaminated soil. The objectives of this study were to i) examine comparative efficiency of PBR, PM and BGR alone or in combined form to improve maize biomass and physiology and ii) understand the role of organic acid production in the root exudates of maize for Cd accumulation and translocation.

2. Materials And Methods

2.1 Source of PBR, PM and BGR

Plant beneficial bacterial strain *Pantoea* sp. WP-5 (accession no. HE661627; Tahir et al., 2015) obtained from NIBGE Biotech Resource Centre. Recently this strain was characterized positive for IAA, produced organic acids and solubilized P in culture medium (Tahir et al., 2020). The organic manures such as PM and BGR collected from local industry were characterized for nutritional status using the standard procedures. The PM contains 2.13% N, 1.91% P and 1.52% K, while the BGR contains 1.71% N, 0.96% P, 1.26% K.

2.2. Cd tolerance measurement of PBR

The plant beneficial bacterial strain *Pantoea* sp. WP-5 was tested for Cd tolerance through measuring the minimum inhibitory concentration (MIC) of Cd. The strain was grown in nutrient-agar medium amended with 0-2000 mg L⁻¹ Cd using CdCl₂ as salt. MIC for the Cd was calculated by observing the growth of the strain after 7 days of incubation at 28 ± 2 °C. The minimum concentration on which the growth of the strain *Panotea* sp. WP-5 was inhibited, considered as MIC.

2.3. Pot experiment

A pot experiment was carried out at Agriculture research farm of Bahauddin Zakariya University Multan (30°11'52"N 71°28'11"E), Pakistan. For this purpose, soil was collected from an experimental field of the Agriculture research farm of Bahauddin Zakariya University Multan from the depth of 0-15 cm using auger. The soil was dried up in air, minced and sieved through a 2 mm sieve. A composite soil sample before pot experimentation was examined for physico-chemical characteristics (saturation percentage, total nitrogen, organic matter, soil texture, available phosphorus, extractable potassium, total Cd, electrical conductivity and pH) by following standard analytical methods as mentioned in ICARDA manual. The proportion of sand, silt and clay in the soil was 25.6%, 53.1% and 18.4% respectively. The pH, saturation percentage (SP) and electrical conductivity (EC) values were estimated as 8.2, 30%, and 3.41 dS m⁻¹, respectively. The organic matter (OM), total N, available P, extractable K and Cd contents values were measured as 0.6%, 0.04 mg kg⁻¹, 6.2 mg kg⁻¹, 198 mg kg⁻¹ and 0.2 mg kg⁻¹, respectively.

The homogenized soil (10 kg) was filled in earthen pots of area 0.035 m². The soil was spiked with 75 mg Cd kg⁻¹ soil using CdCl₂ as salt five days prior to start of the experiment. After five days of spiking, all the treatments (PBR, PM, BGR, PBR+PM, PBR+BGR, PM+BGR, PBR+PM+BGR) along with control without PBR, BGR and PM) were applied in Cd contaminated (75 mg Cd kg⁻¹ soil) and normal soil. The manures PM and BGR were applied @ of 0.5 kg/pot. To inoculate the PBR strain (*Pantoea* sp. WP-5), the seeds of maize hybrid ICI-9091 (obtained from Ayyub Agriculture Research institute, Faisalabad) were mixed with mixture of over-night grown bacterial culture of *Pantoea* sp. WP-5 with strength of 10⁹ colony forming units (cfu) per mL and the sugar cane filter mud (at the rate of 0.5 g per 100 g seed). The seeds (10 seeds per pot) were sown in the earthen pots on March 20, 2017. Crop was fertilized with NPK at 200, 150 and 150 kg ha⁻¹, respectively using urea (NH₂)₂CO, diammonium phosphate (NH₄)₂HPO₄ and SOP (K₂SO₄) as source of N, P₂O₅ and K₂O, respectively. Completely randomized design (CRD) was used in laying out the experiment. Five replications (n=5) were used for each treatment. Thinning was done at 20 days after

sowing and three seedlings was maintained in each pot. A standard criterion for irrigation was followed (Ayer's and Westcot, 1985). Maize crop was harvested manually on May 20, 2017.

2.3 Data collection

2.3.1 Germination count (%) at 8 days after sowing

After the eight days of sowing, germinating seedlings were counted, and germination percentage was noted by using the following formula:

$$\text{Germination count (\%)} = \frac{\text{Total number of germinated seedlings}}{\text{Total number of seeds sown}} \times 100 \quad (1)$$

2.3.2 Physiological parameters and enzymatic analysis

Fresh leaf samples of maize were obtained at 40 days after sowing to measure the chlorophyll a & b contents, carotenoids contents, antioxidant enzyme activity, photosynthetic rate, stomatal conductance, electrolyte leakage and relative water contents in leaves.

2.3.2.1 Chlorophyll content determination

Chlorophyll contents were measured by grinding the 250 mg of fresh leaf samples in liquid nitrogen. A 10 mL volume of acetone/water (80/20: v/v) were added in the sample and kept at 4°C for 24 h. After the 24 h, the samples were centrifuged at 10000 *g* for 10 min and the supernatant was collected in falcon tubes. Absorbance of the samples was recorded at 470, 646 and 663.2 nm. Chlorophyll contents were determined by using the procedure adopted by Lictenthaler (1987).

2.3.2.2. Antioxidant enzyme analysis

For the analysis of antioxidants enzymes, fresh leaves of maize were minced in liquid nitrogen. The grinded sample was homogenized in phosphate buffer (0.05 M and pH=7.1). The homogenized sample was filtered and centrifuged at 16000*g* for 10 min at 4 °C. By adopting the procedure designated by Aebi (1984), activity of catalase enzyme was measured. In this procedure, the enzyme extract was mixed with 300 mM H₂O₂, phosphate buffer (50 mM) and CA (2.0 mM, pH 7.0). Due to H₂O₂ disappearance ($\epsilon \frac{1}{4} 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$), diminution in absorbance at 240 nm was measured.

Activity of ascorbate peroxidase (APX) enzyme was measured using the method described by Nakano and Asada (1981). For this method, 100 μL enzyme extract was mixed with equal volume of 7.5 mM ascorbate and 300 mM H₂O₂, 2.7 mL of 25mM potassium phosphate buffer and 2.0 mM ethylenediaminetetraacetic acid (C₁₀H₁₆N₂O₈) (neutral pH). To measure the ascorbate oxidation activity, wavelength at 290 nm was noted.

Peroxidation of lipids in leaf tissue is represented by malondialdehyde (MDA) contents and this was measured by using thiobarbituric acid-reactive-substances assay (TBARS; Heath and Packer, 1968). For this, 0.25 g of fresh leaves were mixed with trichloro acetic acid (TCA) and centrifugation was done at 12000g for 15 min. The supernatant was separated, and 1.0 mL of the supernatant was mixed with 4.0 mL of 20% TCA comprising 0.5% thiobarbituric acid (TBA). The mixture was placed at 95 °C for 30 min. After the heating, it was rapidly cooled in an ice bath and centrifuged at 10,000 g for 10 min. The supernatant was analyzed on spectrophotometer at 532 nm and value for nonspecific absorbance at 600 nm was deducted. Using extinction coefficient of 155 mM⁻¹ cm⁻¹, the calculation of MDA contents was made.

2.3.2.3. Photosynthetic rate and stomatal conductance

Stomatal conductance and rate of photosynthesis were measured on portable Infra-red Gas Analyzer (IRGA; LCA-4; Analytical Development Company, Hoddeson, UK) by using the condition reported previously (Ben-Asher et al., 2006). Fully expanded flag leaves of maize were used to note the IRGA readings at a specific time i.e. at 10:00 to 11:00 a.m.

2.3.2.4. Relative water content and electrolyte leakage

Method of Mayak et al. (2004) was used to measure the relative water contents (RWC) in maize. Fresh leaves of maize were taken and weighed to get leaf fresh biomass. The leaves were then placed in water for overnight. After that, weight of the leaves was noted (i.e. fully turgid weight). To measure the oven dried biomass, the fully turgid leaves were dried in an oven at 70±2 °C for 48 h.

$$\text{RWC} = \frac{\text{fresh biomass} - \text{dry biomass}}{\text{plant turgid biomass} - \text{dry biomass}} \times 100 \quad (2)$$

The method described by Ahmad et al. (2014) was used to measure the electrolyte leakage (ELL). In this method, completely extended flag leaf was cut into pieces of about 5 mm length. These pieces were placed in test tubes filled with 10 mL double distilled water. The tubes were kept on shaking at 150g and 30 °C for 4 h. After that, the electrical conductivity (EC1) of the medium was noted. Then all the test tubes were autoclaved by placing them in an autoclave and cooled down to 25 °C. Afterwards, electrical conductivity (EC2) of the cooled samples was measured. ELL was calculated by:

$$\text{Electrolyte leakage (ELL)} = \frac{\text{EC1}}{\text{EC2}} \times 100 \quad (3)$$

2.3.3 Measurement of growth data

Maize plants were harvested on May 20, 2017 i.e. at 60 days after sowing. At the time of harvesting, maize plants were uprooted, and roots were separated from each plant. The separated roots were washed in tap water and placed separately on a transparent polyethylene sheet. The sheet along with the roots were placed on desktop scanner. The roots were scanned, root image was created on computer and the root length was measured using root image analysis program. Shoot length of each plant was measured with measuring tape. At the time of harvesting, fresh weight of both root and shoot were measured separately with the help of laboratory weighing balance. After measuring the fresh weight, root and shoot were kept in an oven at 70 °C for 48 h and weighed separately on digital balance to measure dry weight.

2.3.4 Organic acids detection in root exudates of maize

Root samples were collected from each treatment at flag leaf stage. The roots were placed in 30 mL sterilized distilled water and kept on shaking at 150g for 5 days. Thereafter, supernatant of samples was collected through centrifuge at 6000g for 8 min and concentrated to 1.5 mL in a concentrator (Concentrator 5301, Eppendorf, Germany) and filtered over a 0.2 µm filter (Orange Scientific GyroDisc CA-PC, Belgium). To determine the organic acids (acetic acid and citric acid), the filtrates were analyzed by HPLC, using a Perkin Elmer series 200 with 20 µL auto-sampler PE NELSON 900 series interface, PE NELSON 600 series link and Perkin Elmer NCI 900 Network Chromatography interface using Diode-array detector at 210 nm and their UV spectra (190–400 nm), Microgaurd Cation-H Precolumn and an Aminex HPx-87H analytical column for separation. Sulfuric acid (0.001N) was used as mobile phase with flow rate of 0.6 mL min⁻¹. The run was isocratic, and the run time of each sample was 20 min. Solutions (100 µg L⁻¹) of acetic and citric acid (Daejung, Korea) were used as standards.

2.3.5 Cadmium concentration in plant tissue (root and shoot)

The root and shoot samples were kept in oven at 70 °C for 24 h to get oven dried. The dried samples were weighed on digital balance. After weighing, the samples were mashed, and digested with nitric acid (HNO₃) and perchloric (HClO₄) acid (3:1 ratio). The digested samples were placed on a hot plate for heating at 350 °C until the dense white fumes produced. The samples were then cooled, passed through a Whatman 40, and stored. The Cd in the filtered samples was determined on atomic absorption spectrophotometer with detection limit of 0.002 µg L⁻¹ (PerkinElmer, 100 Analyst, Waltham, USA). Translocation factor (TF) was measured according to Ahmad et al. (2014).

2.4 Statistical analysis

Normal distribution ($P > 0.05$) test on all the data collected was performed according to Shapiro-Wilk test. Levene test was performed to verify the homogeneity of variance ($P > 0.05$) of sample data. Statistix v8.1 was used to analyze the data statistically using one-way analysis of variance (ANOVA). The data presented here is the average of five replicates ($n=5$) \pm standard error. Linear regression analysis was applied to determine relation of Cd concentration in maize plant to organic acids concentration in root exudates of maize. When the overall, main effect was significant, treatments mean was further compared using LSD test at $P < 0.05$ probability level (Steel *et al.*, 1997).

3. Results

3.1. Minimum inhibitory concentration of cadmium

The PBR strain *Pantoea* sp. WP-5 showed tolerance up to 1000 mg L⁻¹ of Cd, further increase in Cd concentration inhibited the growth of this strain.

3.2. Pot experiment

3.2.1. Germination count (%) at 8 days after sowing

Analysis of data revealed that BGR treatment gave significantly higher germination percentage (60%) under Cd stress conditions at 8 days after sowing (DAS; Fig. 1a). The treatments MtPBR + BGR, MtPBR + PM and MtPBR + BGR + PM resulted in 57% germination count in Cd contaminated soil. Minimum values of germination percentage i.e. 43% was recorded in control treatment in Cd contaminated soil. Under no Cd stress, the MtPBR + BGR resulted in higher germination count (60%) at 8 DAS (Fig. 1a).

3.2.2. Plant height (cm) and dry weight (g per plant)

Under normal soil conditions, MtPBR + BGR gave higher plant height (104 cm) and plant dry weight (25.9 g per pot) as compared to all other treatments (Table 1). Application of PM, PM + MtPBR and PM + BGR resulted in plants of height 96 cm, 92.8 and 85.5 cm respectively, under normal soil conditions. The treatments PM + BGR and MtPBR + BGR + PM produced 19.6 g pot⁻¹ and 18 g pot⁻¹ plant biomass respectively, under normal soil conditions. In Cd contaminated soil, the treatment (MtPBR + BGR) produced significantly ($P < 0.05$) higher plant height (97.2 cm) and plant dry weight (18.0 g per pot). The treatments MtPBR + PM, BGR + PM and MtPBR + BGR + PM gave plant height 92.3 cm, 86.0 cm, and 87.7 cm respectively, in Cd contaminated soil (Table 1). The treatment MtPBR + PM and MtPBR + BGR + PM produced 15.2 g pot⁻¹ and 15.0 g pot⁻¹ plant biomass respectively, in Cd contaminated soil. Minimum values of these parameters were recorded in control treatment under both the soil conditions.

Table 1

Effect of *Pantoea* sp. strain WP-5 and organic amendments on maize plant height, biomass, and physiology in normal and Cd contaminated soils

Treatments		Plant biomass (g pot ⁻¹)	Plant height (cm)	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal conductance ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$)	RWC (%)
Normal soil	MtPBR	14.1 \pm 0.5d	76.5 \pm 1.0g	22 \pm 0.7bc	3.55 \pm 0.10bc	72 \pm 1.1c
	BGR	11.5 \pm 1e	70.5 \pm 1.0g	21 \pm 0.5c	3.50 \pm 0.12c	70 \pm 1.1d
	PM	2.9 \pm 0.7i	96.0 \pm 1.0b	21 \pm 0.6c	3.43 \pm 0.13cd	71 \pm 1.0cd
	MtPBR + BGR	25.9 \pm 2a	104.0 \pm 1.0a	24 \pm 0.4a	3.83 \pm 0.11a	85 \pm 1.0a
	MtPBR + PM	16.7 \pm 0.8c	92.8 \pm 1.0bc	22 \pm 0.5bc	3.55 \pm 0.11bc	78 \pm 1.2b
	BGR + PM	19.6 \pm 0.5b	85.5 \pm 0.2de	21 \pm 0.5c	3.52 \pm 0.13c	73 \pm 1.3c
	MtPBR + BGR + PM	18.0 \pm 0.5bc	83.0 \pm 3.0de	23 \pm 0.5b	3.65 \pm 0.12ab	80 \pm 1.0b
	Control	1.4 \pm 0.5ij	43.2 \pm 4.0hi	20 \pm 0.5d	3.40 \pm 0.10d	65 \pm 1.1e
Cd contaminated	PGPR	8.0 \pm 0.7f	80.8 \pm 1.0ef	17 \pm 0.4f	2.52 \pm 0.12g	59 \pm 1.0
	BGR	4.7 \pm 0.5h	39.0 \pm 4.0i	16 \pm 0.5g	2.46 \pm 0.15g	58 \pm 1.2h
	PM	0.9 \pm 0.2j	45.6 \pm 1.0h	16 \pm 0.7g	2.22 \pm 0.14h	57 \pm 1.3
	MtPBR + BGR	18.0 \pm 0.5bc	97.2 \pm 1.0b	19 \pm 0.6de	3.00 \pm 0.10e	65 \pm 1.0e
	MtPBR + PM	15.2 \pm 0.5d	92.3 \pm 1.5bc	17 \pm 0.5f	2.42 \pm 0.13gh	61 \pm 1.1fg
	BGR + PM	6.4 \pm 0.5g	86.0 \pm 0.2de	16 \pm 0.5g	2.35 \pm 0.12gh	60 \pm 1.2g

Values are the means (\pm SE) of five replicates (n = 5). Different letters within columns show statistically significant difference ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test. (RWC = relative water contents)

Treatments	Plant biomass (g pot ⁻¹)	Plant height (cm)	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal conductance ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$)	RWC (%)
MtPBR + BGR + PM	15.0 \pm 0.8d	87.7 \pm 0.5cd	18 \pm 0.7e	2.75 \pm 0.11f	63 \pm 1.1f
Control	0.8 \pm 0.4j	41.0 \pm 2.5hi	15 \pm 0.4h	2.00 \pm 0.10i	55 \pm 1.0i
LSD	6.0	7.0	3	0.5	4.0
($P \leq 0.05$)	0.001	0.001	0.005	0.001	0.02
Values are the means (\pm SE) of five replicates (n = 5). Different letters within columns show statistically significant difference ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test. (RWC = relative water contents)					

3.2.3. Physiological parameters of maize

Under normal soil conditions, the treatment MtPBR + BGR increased the photosynthetic rate, stomatal conductance and RWC by 20%, 13% and 31% respectively, over control treatment. Under Cd stress conditions, the treatment MtPBR + BGR showed significantly ($P < 0.05$) higher photosynthetic rate ($19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($3 \mu\text{mol CO}_2 \text{ mol air}^{-1}$) and RWC (65%) as compared to all other treatments (Table 1). However, the treatment MtPBR + BGR + PM increased the photosynthetic rate, stomatal conductance and RWC by 20%, 38% and 15% over control treatment in Cd contaminated soil. Application of MtPBR as sole treatment in normal soil also performed well and increased the photosynthetic rate by 10%, stomatal conductance by 4% and RWC by 11% over the control treatment. While in Cd contaminated soil the MtPBR as sole application increased the photosynthetic rate by 13%, stomatal conductance by 26% and RWC by 7% over the control treatment. Minimum values of these parameters were recorded in the control treatment under normal as well as Cd stress conditions (Table 1).

Under normal soil conditions, the application of PM alone and MtPBR + BGR + PM gave significantly ($P < 0.05$) higher chlorophyll *a* content i.e. 2.07 and 2.02 mg g^{-1} leaf fresh weight respectively, as compared to all other treatments (Fig. 1b). In the Cd contaminated soil, the treatment MtPBR + BGR gave higher chlorophyll *a* content i.e. 1.87 mg g^{-1} leaf fresh weight as compared to other treatments in Cd contaminated soil (Fig. 1b). The control treatment resulted in minimum chlorophyll *a* content under normal as well as under Cd stress conditions.

Application of MtPBR as sole treatment gave significantly ($P < 0.05$) higher chlorophyll *b* content (1.5 mg g^{-1} leaf fresh weight) as compared to other treatments under normal soil conditions (Fig. 1c). After the MtPBR treatment, the treatments BGR, BGR + PM and MtPBR + BGR + PM produced chlorophyll *b* content

0.8, 0.76 and 0.78 mg g⁻¹ leaf fresh weight respectively, under the normal soil conditions. In Cd contaminated soil, the MtPBR as sole treatment gave significantly ($P < 0.05$) higher chlorophyll *b* content (0.95 mg g⁻¹ leaf fresh weight) as compared to the other treatments. After the MtPBR treatments, the treatments BGR, MtPBR + BGR, BGR + PM and MtPBR + BGR + PM also increased the chlorophyll *b* contents by 93%, 73%, 118% and 103% respectively, over the control treatment in Cd contaminated soil (Fig. 1c).

In normal soil, the application of MtPBR as sole treatment resulted in significantly ($P < 0.05$) higher carotenoid contents i.e. 2.01 mg g⁻¹ leaf fresh weight. After the MtPBR treatment, the treatment MtPBR + BGR produced higher carotenoids content (1.13 mg g⁻¹ leaf fresh weight) in normal soil (Fig. 1d). In Cd contaminated soil, the treatment MtPBR + BGR gave significantly ($P < 0.05$) higher carotenoid contents (1.06 mg g⁻¹ leaf fresh weight) as compared to the other treatments. Minimum carotenoids were measured in the control treatments under normal as well as under Cd stress conditions.

Membrane damage was determined by measuring the ELL. It was noted that ELL was significantly ($P < 0.05$) higher in plants grown in Cd contaminated soil as compared to that of the plants in normal soil (Fig. 2a). Among the treatments, the values of ELL were significantly ($P < 0.05$) higher in control treatment i.e. 61.6% in Cd contaminated soil and 40.5% in normal soil. Application of organic amendments either alone or in combined form tended to decrease the ELL value. In normal soil, the treatment MtPBR + BGR reduced the ELL value up to 28.2% while in Cd contaminated soil the same treatment reduced the ELL value up to 45% (Fig. 2a).

3.2.4. Antioxidant enzyme analysis

Effect of Cd doses and organic amendments was observed significant ($P < 0.05$) on APX, catalase and MDA contents in leaves of maize. Analysis of data indicated APX production was higher in plants grown in Cd contaminated soil as compared to that in normal soil. Among the treatments, the control treatment resulted in significantly higher APX concentration (162.0 g⁻¹ fwt and 57.8 g⁻¹ fwt respectively in Cd contaminated and normal soil) in leaves of maize as compared to all other treatments (Fig. 2b). Organic amendments reduced the values of APX in normal and Cd contaminated soil. In normal soil, APX concentration (29.8 g⁻¹ fwt) was recorded significantly ($P < 0.05$) lower in leaves of MtPBR + BGR + PM treated plants. The values of APX in the MtPBR + BGR treated plants was 30.1 g⁻¹ fwt in normal soil. In Cd contaminated soil, the values of APX were 128.2 g⁻¹ fwt and 129.5 g⁻¹ fwt, respectively due to application of MtPBR + BGR and MtPBR + BGR + PM (Fig. 2b). The application of MtPBR as sole treatment gave APX value 132.5 g⁻¹ fwt in Cd contaminated soil and 33.5 g⁻¹ fwt in normal soil.

Catalase activity was recorded higher (80.9 g⁻¹ fwt and 70.8 g⁻¹ fwt, respectively under Cd stress and normal soil conditions) in control treatment as compared to all other treatments (Fig. 2c). Minimum catalase concentration (61.0 g⁻¹ fwt) was recorded in leaves of MtPBR + BGR + PM treated plants grown in Cd contaminated soil. The treatment MtPBR + BGR showed 62.1 g⁻¹ fwt catalase activity in Cd

contaminated soil. While in normal soil, the values of catalase activity were 42.1 and 44.2 g⁻¹ fw, respectively in MtPBR + BGR + PM and MtPBR + BGR treatments. Application of treatments like PM, BGR and MtPBR as sole treatment also performed better. Sole application of MtPBR showed catalase activity 65.3 g⁻¹ fw in Cd contaminated soil and 48.4 g⁻¹ fw in normal soil which were much lesser than control and sole application of PM and BGR.

The control treatment produced significantly higher contents of MDA i.e., 11.6 µM g⁻¹ fw and 6.0 µM g⁻¹ fw, respectively in leaves of plants grown under Cd stress and normal soil conditions (Fig. 2d). Minimum MDA contents i.e. 5.8 µM g⁻¹ fw were recorded in leaves of MtPBR + BGR and 6.2 µM g⁻¹ fw in MtPBR + BGR + PM treated plants grown in Cd contaminated soil. In normal soil, the values of MDA contents were 2 µM g⁻¹ fw in MtPBR + BGR and 3.2 µM g⁻¹ fw in MtPBR + BGR + PM treated plants. The sole application of MtPBR showed MDA contents i.e. 7.1 µM g⁻¹ fw in Cd contaminated soil and 4.2 µM g⁻¹ fw in normal soil which were much lesser than the control.

3.2.5. Number of roots (per plant), root length (cm) and root dry weight (g per plant)

The treatment MtPBR + BGR gave significantly ($P < 0.05$) higher number of roots (20.3 ± 1 roots per plants), root length (40.7 ± 1 cm) and root dry weight (2.80 ± 0.2 g per plant) as compared to control and all other treatments in Cd contaminated soil (Table 2). The BGR application as sole treatment gave number of roots (18 ± 0.5 roots per plant) and root length 40 ± 2 cm which were much higher than control treatment in Cd contaminated soil. Under normal soil conditions, the treatment MtPBR + BGR gave significantly higher number of roots i.e. 29.5 roots per plant, root length i.e. 58.8 cm and dry weight i.e. 4.15 g per plant as compared to all other treatments (Table 2). The treatment MtPBR + BGR + PM produced 26.0 ± 1 number of roots per plant and root dry weight 2.88 g per plant in normal soil. The PM sole treatment gave 53.3 ± 1 root length in normal soil. Minimum values of all these parameters were recorded in the control treatment under both the soils conditions.

Table 2

Effect of *Pantoea* sp. strain WP-5 and organic amendments on root growth and Cd concentration in maize (root + shoot) in normal as well as Cd contaminated soils

Treatments		Number of roots plant ⁻¹	Root length (cm)	Root dry biomass (g plant ⁻¹)	Shoot Cd (mg kg ⁻¹ dwt)	Root Cd (mg kg ⁻¹ dwt)
Normal soil	PGPR	19.5 ± 1.0d	34.2 ± 0.5g	3.01 ± 0.2ab	2.33 ± 0.5hi	6.37 ± 0.5kl
	BGR	12.5 ± 1.0i	33.8 ± 1.0gh	2.06 ± 0.1bc	3.4 ± 0.6ghi	9.52 ± 0.8jk
	PM	20.7 ± 2.0c	53.3 ± 1.0b	0.84 ± 0.1c	5.23 ± 0.3gh	3.33 ± 0.5
	PGPR + BGR	29.5 ± 1.0a	58.8 ± 2.0a	4.15 ± 0.2a	0.50 ± 0.1i	16.07 ± 1.0i
	PGPR + PM	17.0 ± 1.0ef	38.0 ± 2.0ef	2.20 ± 0.1bc	1.33 ± 0.1hi	4.23 ± 0.5
	BGR + PM	15.5 ± 0.3fgh	44.9 ± 1.0c	2.88 ± 0.1ab	2.9 ± 0.2ghi	6.13 ± 0.5kl
	PGPR + BGR + PM	26.0 ± 1.0b	41.5 ± 1.0d	2.98 ± 0.1ab	1.33 ± 0.2hi	12.03 ± 1.2ij
	Control	13.0 ± 0.5hi	31.3 ± 0.5i	0.61 ± 0.1c	6.63 ± 0.5g	2.30 ± 0.5
Cd contaminated	PGPR	14.3 ± 0.2ghi	28.3 ± 0.5jk	2.34 ± 0.1a	108.3 ± 0.5d	159.00 ± 2.5g
	BGR	18.0 ± 0.5def	40.0 ± 2ef	1.38 ± 0.2bc	115.7 ± 0.5c	288.33 ± 3.0c
	PM	13.0 ± 0.5hi	30.0 ± 0.5ij	0.74 ± 0.1c	135.3 ± 1.2b	166.00 ± 1.2f
	PGPR + BGR	20.3 ± 1.0cd	40.7 ± 1.0de	2.80 ± 0.2ab	70.3 ± 1.2f	385.00 ± 2.6a
	PGPR + PM	12.5 ± 0.5i	37.3 ± 0.5f	1.90 ± 0.2bc	108.3 ± 2.0d	213.33 ± 2.3e
	BGR + PM	17.5 ± 0.5ef	26.7 ± 1.2k	1.78 ± 0.2bc	138.3 ± 1.2b	245.33 ± 1.5d
	PGPR + BGR + PM	16.5 ± 1.0fg	31.7 ± 0.5hi	1.72 ± 0.2bc	103.7 ± 1.0e	296.00 ± 3.0b

Values are the means (± SE) of five replicates (n = 5). Different letters within columns show statistically significant difference ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test.

Treatments	Number of roots plant ⁻¹	Root length (cm)	Root dry biomass (g plant ⁻¹)	Shoot Cd (mg kg ⁻¹ dwt)	Root Cd (mg kg ⁻¹ dwt)
Control	13.0 ± 0.5hi	27.5 ± 0.5k	0.50 ± 0.1c	179.7 ± 1.5a	130.67 ± 3.0h
LSD	2.5	2.3	2.0	4.0	6.0
($P \leq 0.05$)	0.0001	0.02	0.02	0.001	0.01
Values are the means (± SE) of five replicates (n = 5). Different letters within columns show statistically significant difference ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test.					

3.2.6. Organic acid concentration in root exudates of maize (µg g⁻¹ root fwt)

Acetic and citric acid concentrations were recorded higher in root exudates of maize plants grown in Cd-contaminated soil as compared to that of normal soil (Fig. 2e & 2f). Among the treatments, the application of MtPBR strain *Pantoea* sp. WP-5 either alone or in combination with BGR and PM resulted in higher secretion of acetic and citric acid concentrations in root exudates of maize (Fig. 2e & 2f). Application of MtPBR + BGR produced significantly ($P < 0.05$) higher amount of acetic acid i.e. 52.7 and 40.5 µg g⁻¹ root fresh weight, respectively in Cd contaminated and normal soil. The treatment MtPBR + BGR + PM produced acetic acid 51.7 µg g⁻¹ in Cd contaminated while 39.8 µg g⁻¹ root fresh weight in normal soil. The sole application of MtPBR produced acetic acid by 39.7 and 30.5 µg g⁻¹ root fresh weight in Cd and normal soils, respectively, which was higher than control treatment under both the soil conditions.

The treatment MtPBR + BGR gave significantly higher amount of citric acid i.e. 22.8 µg g⁻¹ root fresh weight in Cd contaminated soil and 17.5 µg g⁻¹ root fresh weight in normal soil as compared to all other treatments (Fig. 2e & 2f). The values of citric acid produced in the treatment MtPBR + BGR + PM were at par with that of the treatment MtPBR + BGR under both the soil conditions. The sole application of MtPBR produced 16.5 and 12.7 µg g⁻¹ root fresh weight citric acid in Cd contaminated and normal soil, respectively which were higher than that of the control treatment under both the soil conditions.

3.2.7. Cadmium concentration in shoots and roots (mg kg⁻¹ dwt)

Analysis of data revealed that control treatment showed higher concentration of Cd i.e. 179.7 ± 1.5 mg kg⁻¹ dwt in Cd contaminated while 6.63 ± 0.5 mg kg⁻¹ dwt in normal soil in shoots of maize plants (Table 2). Poultry manure application either alone or with the BGR were the treatment after the control treatment that showed maximum concentration of Cd i.e. 135.3 mg kg⁻¹ dwt in Cd contaminated while

5.23 mg kg⁻¹ dwt in shoots of plants grown in normal soil. Minimum concentration of Cd i.e. 70.3 ± 1.2 and 0.50 ± 0.1 mg kg⁻¹ dwt of shoots in Cd contaminated and normal soils respectively, was noted due to application of MtPBR + BGR treatment. The treatment MtPBR + BGR + PM gave Cd concentration 103.7 ± 1 mg kg⁻¹ dwt of shoot in Cd contaminated soil while 1.33 ± 0.2 mg kg⁻¹ dwt in normal soil (Table 2). The sole application of MtPBR gave Cd concentration 108.3 ± 0.5 mg kg⁻¹ dwt in Cd contaminated while 2.33 ± 0.5 mg kg⁻¹ dwt of shoot in normal soils.

The MtPBR + BGR treatment showed higher concentration of Cd in roots i.e. 385 ± 2.6 mg kg⁻¹ dwt in Cd contaminated while 16.07 ± 1 mg kg⁻¹ dwt in normal soil (Table 2). The treatment MtPBR + BGR + PM gave Cd concentrations 296 ± 3 mg kg⁻¹ dwt of root in Cd contaminated while 12.03 ± 1.2 mg kg⁻¹ dwt in normal soil. The sole application of BGR gave Cd concentrations 288.3 ± 3 mg kg⁻¹ dwt of root in Cd contaminated while 9.52 ± 0.8 mg kg⁻¹ dwt in normal soil (Table 2).

3.2.8. Translocation of Cd

Translocation factor (TF) measured based on root and shoot Cd concentrations. The values of TF were recorded maximum i.e. 2.88 ± 0.2 in control treatment (Fig. 3) in Cd contaminated soil. The various organic amendments tended to reduce the TF values. The treatment i.e. MtPBR + BGR showed significantly ($P < 0.05$) lower (0.03 ± 0.001 and 0.18 ± 0.08) TF value (Fig. 3). The treatment MtPBR + BGR + PM also showed reduced values of TF i.e. 0.11 in normal and 0.35 in Cd contaminated soil. The sole application of BGR gave lesser TF values i.e. 0.36 in normal and 0.40 in Cd contaminated soil but these values were higher than that of the treatment MtPBR + BGR and MtPBR + BGR + PM.

3.2.9. Correlation analysis

Cd concentration in shoots of maize was significantly negatively correlated with respective maize root exudates concentrations of citric and acetic acid in normal (Fig. 4a: $R^2 = 0.81$; 4e: $R^2 = 0.87$) and Cd contaminated soil (Fig. 4c: $R^2 = 0.86$; 4g: $R^2 = 0.90$). Similarly, root Cd concentration was significantly and positively correlated with respective maize root exudates concentrations of citric and acetic acid in normal (Fig. 4b: $R^2 = 0.80$; 4f: $R^2 = 0.92$) and Cd contaminated soil (Fig. 4d: $R^2 = 0.83$; 4h: $R^2 = 0.86$).

4. Discussions

The plant-beneficial rhizobacterial strain *Pantoea* sp. WP-5 is positive for IAA, phosphate solubilization and organic acid production (Tahir et al., 2020), here in this study, it was used as bioinoculant to maize grown in normal as well as in Cd contaminated soil because WP-5 strain showed Cd tolerance up to 1000 mg Cd L⁻¹ in a growth medium. These properties could enable this strain WP-5 to sustain in Cd stress condition because bacterial produced organic acids (acetic acid, citric acid and gluconic acid) have number of functional groups like carboxylic group (-COOH) that might have caused displacement of Cd through formation of either complexes or chelates with (Cd)²⁺ cations in solution (Bali et al., 2020; Mostofa et al., 2013; Potysz et al., 2017). In the present study, the increased concentration of acetic and

citric acids in the root exudates of plants treated with MtPBR + BGR was due to the fact that the BGR helped the bacteria in multiplication and functioning which directly enhanced the organic acid secretion in root exudates of maize plants. The BGR role in improving bacterial multiplication and functioning in rhizosphere has been reported (Abubaker et al., 2012; Tahir et al., 2018). Concentration of Cd in roots of maize was higher in MtPBR inoculated treatment either alone or in combination with BGR under both the soil conditions in our study. Maize roots have potential to uptake Cd from soil and accumulate in roots (Puertas-Mejia et al. 2010; Ahmad et al., 2020) and the relationship between the Cd accumulation in roots and organic acids in root exudates has been reported (Najeeb et al., 2011). Our results indicated that MtPBR application increased the concentration of organic acids in root exudates of maize and the organic acids played a role in complexation, speciation, detoxification, and accumulation of Cd in roots of maize. Our results are in line with that of previous studies which reported the role of organic acids in Cd extraction from soil, Cd speciation and detoxification (Mostofa et al., 2013; Potysz et al., 2017; Bali et al., 2020). Uptake and accumulation of Cd in roots of maize (Puertas-Mejia et al., 2010; Ahmad et al., 2020) and the positive relationship of this trait with the organic acid concentration in root exudates of maize has been reported (Najeeb et al., 2011). Increased concentration of Cd in roots of maize due to PBR application has been reported (Moreira et al., 2014; Rizwan et al., 2016). Correlation analysis further confirmed our results which showed the significant positive relationship ($R^2 = 0.9$ and 0.8 respectively, in Cd contaminated and normal soil; $P < 0.05$) between Cd concentration in roots and the organic acid (acetic acid and citric acid) concentration in root exudates of maize. Translocation and concentration of Cd in shoots was recorded lower in MtPBR and MtPBR + BGR treatment as compared to control treatment in the present study. While the Cd translocation and concentration was higher in shoots of PM treated plants as compared to MtPBR + BGR treated plants in the present study. This indicated that the presence of MtPBR and BGR restricted whereas PM promoted the translocation of Cd to shoots. Results of some studies showed the increased translocation of Cd from roots to shoots particularly in stem due to application of organic manures, but this depends upon the nature and type of manure (Rizwan et al., 2016). Correlation analysis indicated that the significant negative correlation ($R^2 = -0.9$ and -0.8 respectively, in Cd contaminated and normal soil; $P < 0.05$) exist between the organic acid concentration in root exudates and Cd concentration in shoots of maize. Progressive reduction in TF factor of Cd in maize when exposed to MtPBR + BGR treatment was observed in our study and has been reported previously (Ahmad et al., 2018).

Electrolyte leakage, MDA contents and antioxidant enzymes like APX and catalase activity was recorded higher in plants grown in Cd contaminated soil as compared to that of normal soil. Under the Cd contaminated soil conditions, plants faced osmotic stress that caused membrane damage resulting in increased ELL, MDA, APX and catalase contents. The values of these parameters were decreased by the application of organic amendments like MtPBR and BGR either alone or in combine form in the present study. The decrease in ELL, MDA, APX and catalase contents in PBR and BGR treatments indicated that these treatments tended to mitigate the Cd stress effects on plants. This was clearly due to the fact that the application of these treatments increased the organic acids production (acetic and citric acid in root exudates) in maize roots which resulted in increased Cd extraction from soil, Cd concentration in roots

and restricted the translocation of Cd to shoots (low TF values). This ultimately resulted in the reduced Cd toxicity to maize plants and decreased the values of ELL, MDA, APX and catalase contents. Our results are similar with previous studies (Ahmad et al., 2018) who reported the increased ELL, MDA, APX and catalase level in plants under metal stress and the application of organic amendments tended to decrease the values of these parameters (Ahmad et al., 2018). These mechanisms enable the maize plants to cope with Cd stress and resulted in increased values of plant physiological parameters like stomatal conductance, photosynthetic rate and leaf relative water contents of maize in our study, and confirmed the previous findings (Ahmad et al., 2018). Plant growth parameters like plant height, number of roots, root length, root dry matter and plant dry matter were increased due to PBR and BGR application either alone or in combine form over control treatment in our study. This might be due to the fact that the application of PBR and BGR improved the concentration of Cd in roots of maize but restricted its translocation to shoot. The restricted translocation of Cd to shoot caused in lower/no toxicity of Cd in maize, which resulted in decreased membrane damage, increased photosynthetic activity, plant growth and dry matter yield in the present study. Increased plant growth and dry matter yield due application of organic manures in Cd contaminated soil have been reported in previous studies (Ahmad et al. 2015; Putwattana et al. 2015). Similarly, increased uptake of Cd from soil and accumulation in roots and improved maize growth as well as dry matter yield due to application of plant growth promoting rhizobacteria has been reported in previous studies (Ahmad et al., 2015; Sangthong et al., 2015; Moreira et al., 2014).

Application of PM resulted in decreased plant growth and dry matter yield in our study while Shumba et al. (2014) reported an increase in dry matter yield of maize due to PM application in sandy soil. This might be due to higher translocation of Cd to shoot by the PM, which inhibits photosynthetic activity and thus reduced the dry weight of maize plant. We have tried to find the role of bacterial produced organic acids in Cd concentration in roots and its translocation to shoot of maize. Positive correlation between organic acid contents in root exudates and Cd uptake in roots while the negative in case of shoots strengthen our hypothesis that organic acid production in the root exudates of maize remediate Cd in soil. Positive relationship of organic acids in root exudates and Cd uptake by roots has already been reported (Najeeb et al., 2011).

5. Conclusions

It is concluded that the application of organic acid producing MtPBR either alone or in combination with BGR ameliorate Cd contamination in soil through stabilization in maize roots and restrict translocation to shoot, which improve maize biomass and physiology. Therefore, it is conferred that this technique may be used to grow maize in Cd contaminated soil without compromising its biomass yield and quality. Further, the application of PM alone or in combination with BGR increased the Cd translocation to shoot. It is obvious from results that organic acid production in maize root exudates is responsible for stabilization of Cd in roots and translocation to shoot. The high production of organic acids in response to MtPBR + BGR increased whereas low production in response to PM decreased Cd concentration in root.

Based on our findings, we can recommend combined application of MtPBR + BGR for improving maize biomass and phyto-management of Cd contaminated soil.

Declarations

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Authors contributions

MT, IA, AD: writing - original draft preparation, methodology, investigation; UK, MA, MK: methodology, resources & analysis; MS, MBK: writing- reviewing and editing; MT, IA: conceptualization, resources, supervision.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

This study does not involve any humans or animals during experimentation, so it does not applicable in this study.

Consent for publication

This study does not contain data from any individual person, please state so it does not applicable in this section.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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Figures

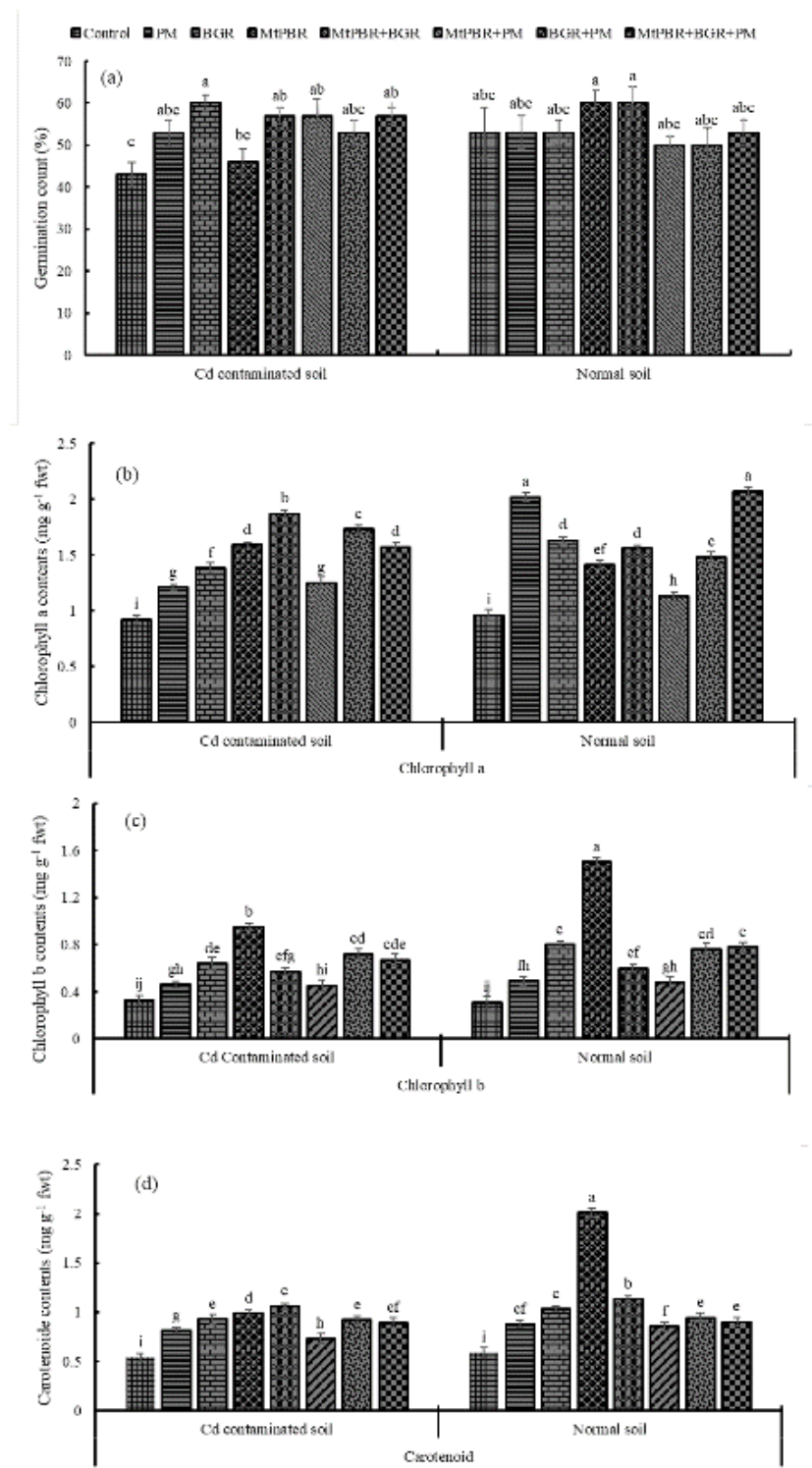


Figure 1

Effect of *Pantoea* sp. strain WP-5 and organic amendments on maize (a) seed germination, (b) chlorophyll a, (c) chlorophyll b and (d) carotenoids contents in normal and Cd contaminated soils. Values are the means (\pm SE) of five replicates ($n=5$). Different letters on bars show statistically significant difference among the treatments ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test.

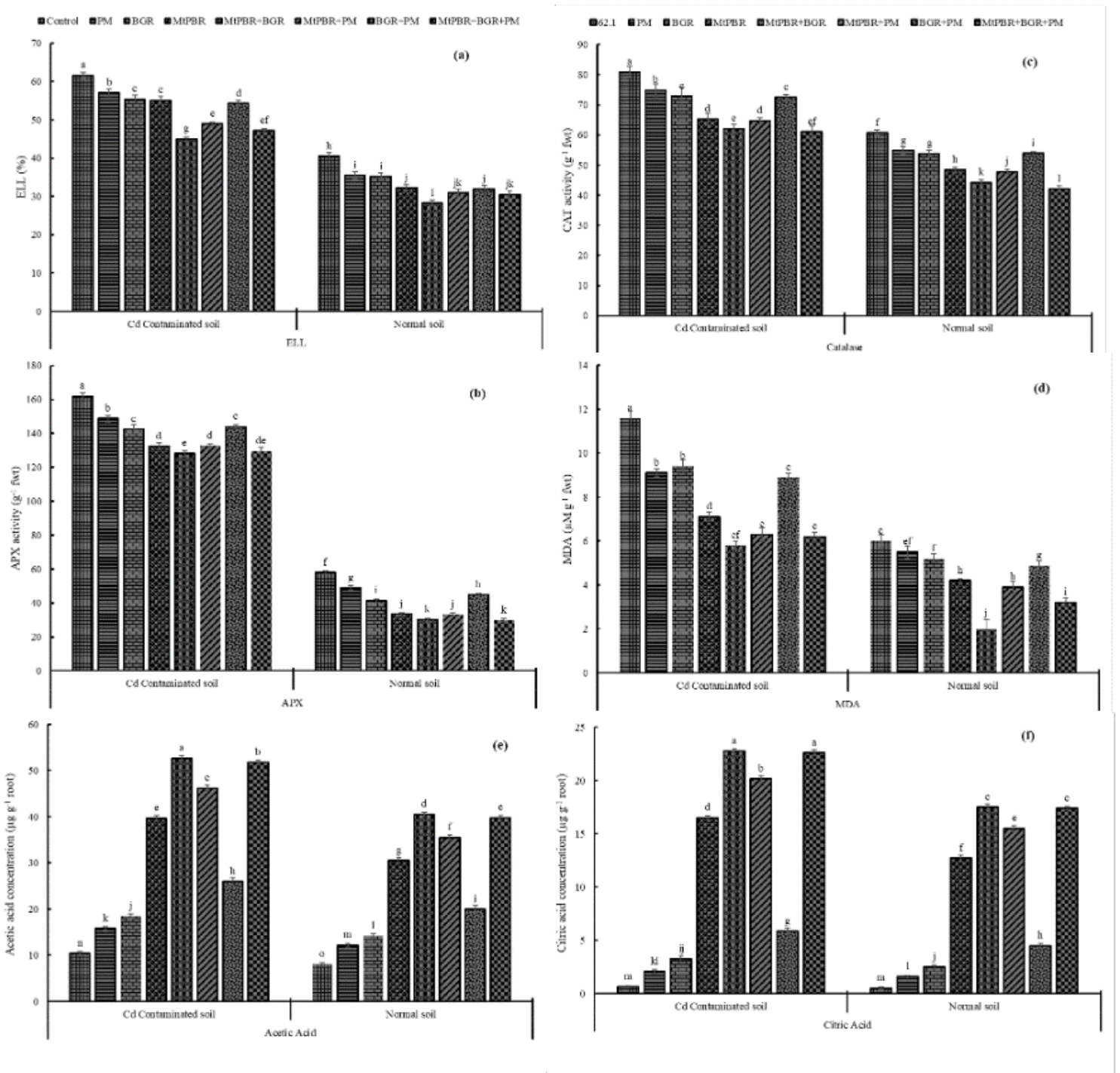


Figure 2

Effect of *Pantoea* sp. strain WP-5 and organic amendments on maize (a) electrolyte leakage (ELL), (b) ascorbate peroxidase (APX), (c) catalase (CAT), (d) malondialdehyde (MDA) contents, (e) acetic acid and (f) citric acid concentration in normal and Cd contaminated soils. Values are the means (\pm SE) of five

replicates (n=5). Different letters on bars show statistically significant difference among the treatments ($P \leq 0.05$) while the values sharing the same letter are statistically non-significant following least significant difference (LSD) test.

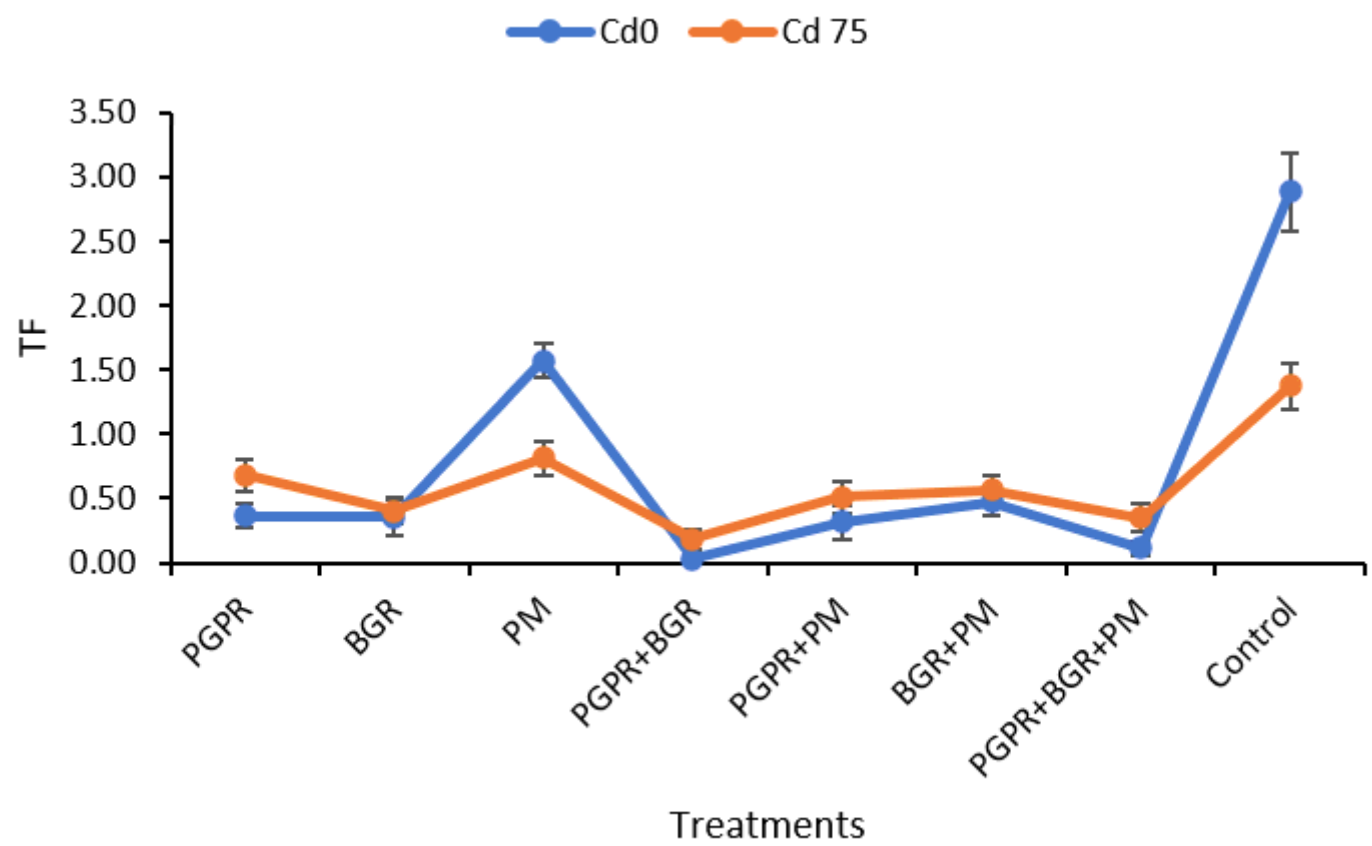


Figure 3

Effect of *Pantoea* sp. strain WP-5 and organic amendments on translocation factor from root to shoot in maize. Values are the means (\pm SE) of five replicates (n=5).

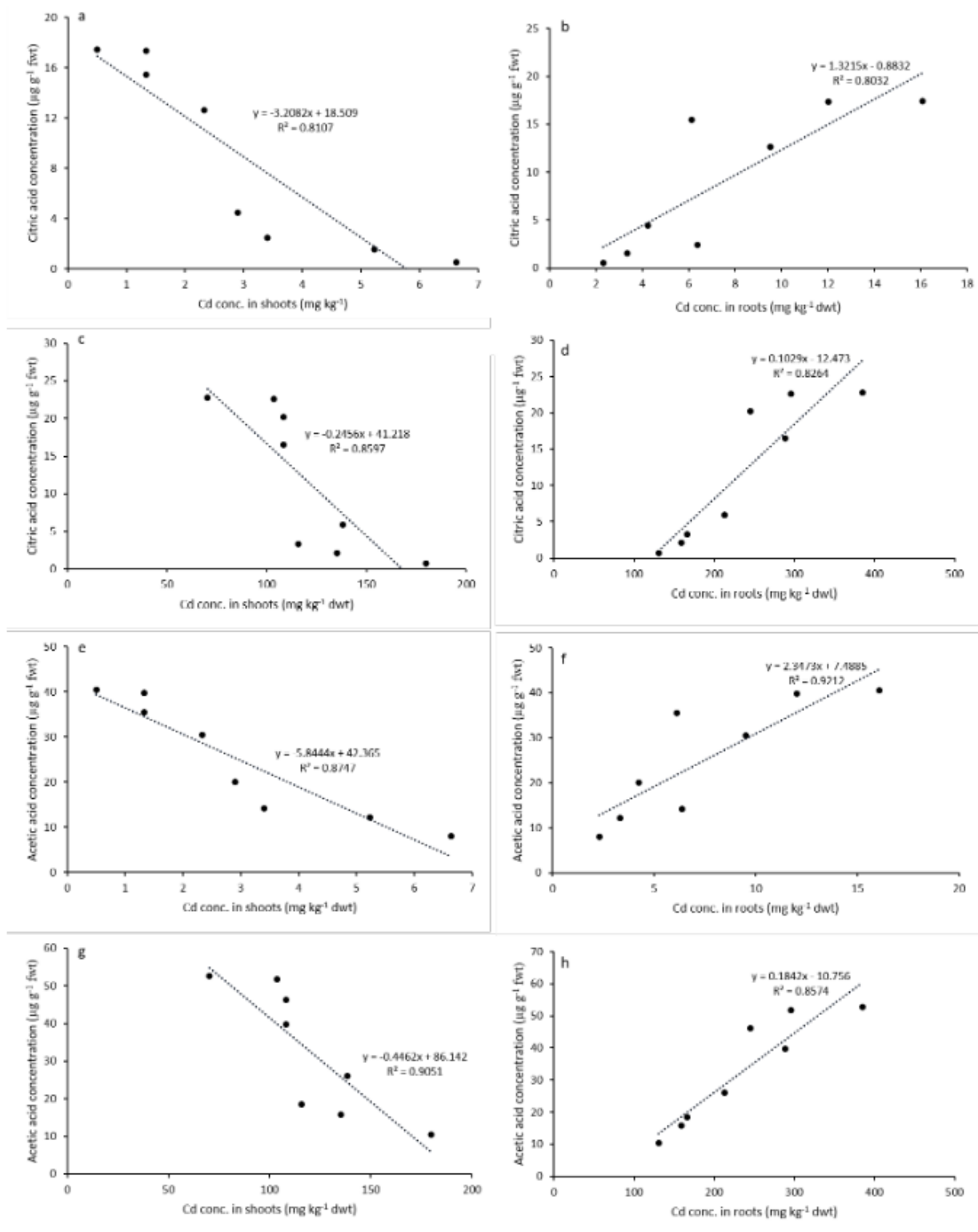


Figure 4

Linear regression analysis to show the relationship of organic acids production with Cd concentrations in shoots and roots of maize in normal (a, b, e, f) and Cd contaminated soils (c, d, g, h) at $P < 0.05$.